Full Length Research paper

Does the ESR-adjusted serum ferritin concentration predict iron deficiency better?

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There has always been a need to develop simple and accurate techniques to improve the predictive value of the serum ferritin concentration (sFC) in patients with systemic inflammation and malignancies. This prospective study examined the impact of adjusting sFC for acute phase reactants with the erythrocyte sedimentation rate (ESR), using the bone marrow aspirate Perl's stain as a gold standard for comparison. Sixty patients (male/female = 35/25), with a mean age of 44 ± 4 years were recruited. Thirty-four of the patients had HIV infection, twenty had haematologic malignancies, four had unexplained severe anaemia, while two had hyperreactive malarial splenomegaly. Haematologic parameters including ESR, ELISA-based serum ferritin concentration (sFC) and bone marrow aspiration (and trephine biopsy, if aspirate was aparticulate) were examined. The effect of ESR-adjustment of the sFC on the iron status predictive value was studied. The difference in the sFC of bone marrow aspirate (BMA)-negative and BMA-positive patients was significant, and was further enhanced with ESR adjustment of the sFC. This study has shown clearly that the predictive value of sFC for assessing iron status can be enhanced using the ESR, in patients with chronic inflammatory conditions and malignancies.

Keywords: Iron, ferritin, bone marrow aspiration, bone marrow trephine, Perl's stain, Nigeria.

INTRODUCTION

Iron deficiency is the commonest cause of anaemia world-wide; although the incidence is higher in developing countries. Though informative, the presence of significant red cell hypochromia and microcytosis are inadequate to predict iron insufficiency. This is largely because several disorders (such as lead poisoning, anaemia of chronic disease, sideroblastic anaemia, etc) which could make even adequate body iron unavailable to the red cells could present similarly. Serum ferritin concentration (sFC) have proved sufficiently accurate, specific and sensitive.(Milman et al. 1997; Schettini et al. 1987; Zemelka and Biesalski 2001) However, its utility as a non-invasive alternative to assess iron status is undermined in the presence of acute-phase reactions.(Sheehan et al. 1978).

Improving the predictive utility of the sFC in the assessment of iron status has been a challenge in most resource-poor centres, where incidentally iron deficiency anaemia is commoner. Therefore, the identification of blue-staining haemosiderin pigments in a Perls'-stained bone marrow biopsy remains the gold standard.(Milman et al. 1997; Mockenhaupt et al. 1999; Odunukwe et al. 2000; Stuart-Smith et al. 2005) However, technical inadequacies and cost have made even this and other non-invasive alternatives unattractive in developing countries. Therefore, in the presence of inflammatory conditions that may lead to an over-estimation of the sFC, there is a need for techniques or other tests to correct for the effect of these confounders. In the past, various candidate tests have been used with variable success; including the erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP).(Kurer et al. 1995; Witte et al. 1988) There is sufficient evidence to suggest that the CRP is a more sensitive and accurate test.(Kurer

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et al. 1995) However, in developing countries, the ESR is more readily available and affordable.

Consequently, we sought to evaluate the effect of sFC correction with ESR in the face of inflammation and malignancies.

PATIENTS, MATERIALS AND METHODS

This prospective study involved consenting adult patients requiring bone marrow studies. The study proposal was approved by the local hospital ethics committee.

Sixty consecutive patients ($\geq$ 16 years) seen from May to November 2006 were enrolled. Written informed consent was obtained from each participant (parent or guardian, in the case of participants < 18 years old) at enrolment. Patients were excluded if they have already commenced therapeutic iron tablets; had received blood transfusions within 4 weeks of enrolment; diagnosed with haemolytic anaemias, iron overload and acute or chronic liver disease; or failed to give consent.

The following tests were conducted: blood cell counts and morphology, ESR, serum ferritin levels, bone marrow aspiration and, in the absence of marrow fragments, bone marrow trephine biopsy. Venous blood, anticoagulated with ethylene-diaminetetraacetic acid (EDTA), was used for cell counts. These were frozen at –30℃ for serum ferritin measurement.(Bain and Bates 2002) Cell counts and ESR were done manually(Bain and Bates 2002) and/or using an automated haematology analyser (ADVIA-60, Bayer HealthCare).

Bone Marrow Aspiration, Trephine Biopsy and Processing

Bone marrow aspirates (BMA, 0.1 - 0.3 ml) were obtained from the iliac crest, and used to make smears. The wet slides were immediately examined for fragments to determine the need for a trephine biopsy. The labelled slides were stored until they were stained using the same batch of potassium ferrocyanide. The study slides were stained in 10 g/l potassium ferrocyanide (diluted in 0.2M hydrochloric acid) for 10 min, washed and counterstained with 0.1% aqueous safranin.(Bain et al. 1992; Hughes et al. 2004) They were all examined using the same microscope, at the same sitting.

For trephine biopsies, 0.8 to 1.2 cm of core bone marrow was obtained using a Jamshidi needle, and fixed in 5% formaldehyde. The samples were decalcified with formic acid according to standard procedures(Baker et al. 2001) and stained with haematoxylin and eosin. Assessment of all the trephine slides were done at the same time, independent of the BMA slides (by the third author). They were examined for golden yellow haemosiderin deposits within the marrow spaces. A modified grading system of Rath and Finch(Rath and Finch 1948) was used. As oil objective was not utilised, grade 1 was determined by the presence of occasional small iron particles in reticulum cells.

Serum ferritin concentrations were determined using a human ferritin enzyme-linked immunosorbent assay (ELISA) Kit (Bio-Quant Inc., San Diego, USA) in one batch. After passing the samples and standards through the ELISA reactions, their optical densities (OD) and concentrations were then read with a microplate ELISA reader (Model 550; Bio-Rad Laboratories, Hercules, USA) at 450 nm, according to the manufacturer's instructions.(Bio-Rad 2005) Ferritin standards supplied by the manufacturer were used to construct a standard curve, from which the sFC of patient samples were determined. ESR-corrected serum ferritin was calculated according to a previously published formula thus: ESR-sFC = 1.311(sFC)

- 0.6213(ESR) - 12.17.(Witte et al. 1988)

Grading of Bone Marrow Iron Content

The haemosiderin content of the marrow slides were graded according to a previously published grading scheme.(Bain et al. 1992; Rath and Finch 1948) First, the number of fragments was counted to a maximum of 20 fragments. Each slide was graded for iron content, after inspecting at least the first 10 fragments in at least 2 slides for the typical Prussian blue-staining deposits. In the positive slides, the number of fragments inspected before a positive fragment was found was noted.

Data Collection and Analysis

Statistical analyses were done with Microsoft Excel and SPSS 11.0 statistical packages.

RESULTS

Patients characteristics

Sixty patients were enrolled (see details in Table 1). The summary of the blood counts show that the haematocrit was borderline. The mean leucocyte count is relatively high, with a wide range. The mean and range of the platelet counts were unremarkable.

Serum ferritin concentration

The average serum ferritin concentration (sFC) was 512 ± 87 ng/l. The concentrations of the samples were automatically determined by the ELISA reader.

Bone marrow aspirate Perl's stain

Of the 59 marrow aspirates assessed for iron content, 53 (90%) had $\geq$ 1 fragment(s), 33 (56%) had $\geq$ 7 fragments, while six (10%) had no fragment. Grades 1-4 positivity was noted in 35 aspirates; none achieved grades 5 or 6. An average of four fragments was examined before a positive fragment was found. The remaining 18 aspirates had no stainable iron. Hughes et al had shown that aspirates with < 7 fragments could not be reliably assessed to be negative.(Hughes et al. 2004) Consequently, 10 of these 18 that had < 7 fragments were not compared with the serum ferritin.

Effect of variables on serum ferritin concentration

Analysis of the serum ferritin results showed that the sFC of anaemic patients (haematocrit $\leq$ 0.30) was significantly

Table 1. Patients' characteristics and haematologic parameters

	No.	Mean	SD	95% CI
Patients' age (year)	60	44	14	40 – 48
Patients' gender	No.	(%)		
Male	35	58.3%		
Female	25	41.7%		
Haemogram	No.	Mean	SD	95% CI
PCV (%)	60	32	8	30 – 34
WBC (x 10^9/L)	60	23.56	64.07	8.6 - 38.6
Platelet (x 10^9/l)	60	203	121	171 – 236
Serum ferritin	No.	Mean	SD	95% CI
sFC (ng/ml)	60	512	343	425 - 598.4

SD = standard deviation, CI = confidence interval, PCV = packed cell volume, WBC = white blood cell count, OD = optical density, sFC = serum ferritin concentration

Table 2. Serum ferritin according to BMA Perl's staining

	No.	Mean	SD	95% CI	T-test
PS = 0	8	284.7	363.8	32.6 - 536.7	0.039
PS ≥ 1	35	495.8	282.8	402.1 - 589.5	
PS=1	12	513.6	387.8	294.2 - 733.1	
PS=2	14	477.3	241.8	350.6 - 603.9	
PS=3	8	541.3	159.3	430.9 - 651.7	
PS=4	1	176.9	na	na	

*ESR-corrected serum ferritin concentration vs. BMA Perl's staining

	No.	Mean	SD	95% CI	T-test
PS=0	5	93.2	80.1	23 - 163.4	0.007
PS≥1	28	537.7	370.9	400.3 - 675.1	

PS = Perl-stainable iron, SD = standard deviation, CI = confidence interval, na = not applicable. *Derived using the formula published by Witte DL et al; Am J Clin Pathol. 1988;90(1):85-87.

higher than that of the non-anaemic (p = 0.017). There is a significant correlation between the sFC of truly negative aspirates (Grade 0 aspirates with ≥ 7 fragments, n = 8) compared with those of positive aspirates (Grades 1-4, n = 35; p = 0.039; Table 2). The level of significance was further enhanced (p = 0.007; Table 2) after adjusting the sFC for ESR, using a previously published formula.(Witte et al. 1988)

From this cohort, grade 0 aspirates can be predicted with 100% and 95% confidence only when sFC ≤ 33 ng/l and ≤ 402 ng/l respectively; while grade ≥ 1 positive (iron-replete) is likewise predictable when sFC ≥ 995 ng/l and ≥ 537 ng/l respectively (Figure 1). Following ESR-correction, the overlap of the sFC ranges for positive versus negative iron status was lost (Figure 2). It was thus possible to predict with 100% confidence iron-replete patients when ESR-corrected sFC (z-sFC) is ≥ 402 ng/l; and iron-depleted patients when z-sFC ≤ 174 ng/l. This information was then used to modify a previously published algorithm (Figure 3) (Hughes et al. 2004)

DISCUSSION

The mean sFC in this study, though expectedly higher than that of the normal healthy population, is surprisingly low compared to that of patients with rheumatoid arthritis, systemic lupus erythematosus (SLE),(Lim et al. 2001) or even Gaucher's disease(Morgan et al. 1983) where concentrations frequently reach the thousands.

Our study further reinforces the fact that the serum ferritin concentration can be used to screen for iron-deficiency, even in patients with co-morbidities; though the predictive value is known to be limited in such settings. The fact that serum ferritin is an acute-phase-reactant has been long established. In this study too, we found a rather wide range within which the iron status is indeterminate, raising the possibility that inflammatory influences have caused wide variations. In the face of these limitations, some authors have examined the erythrocyte sedimentation rate (ESR) as an easy but reasonably accurate way of correcting for the effect of acute phase reactions.(Witte et al. 1988) Others have
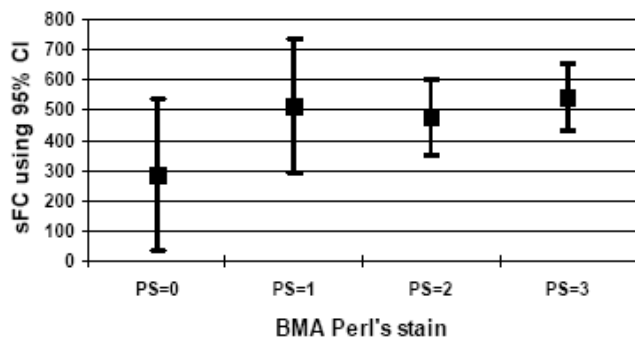
Figure 1. Serum ferritin concentration at increasing levels of BMA stainable iron.
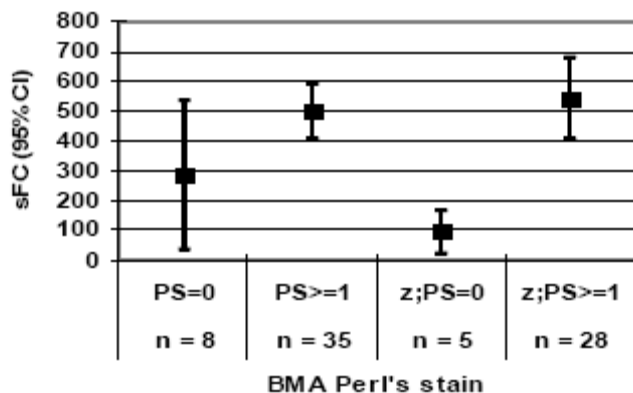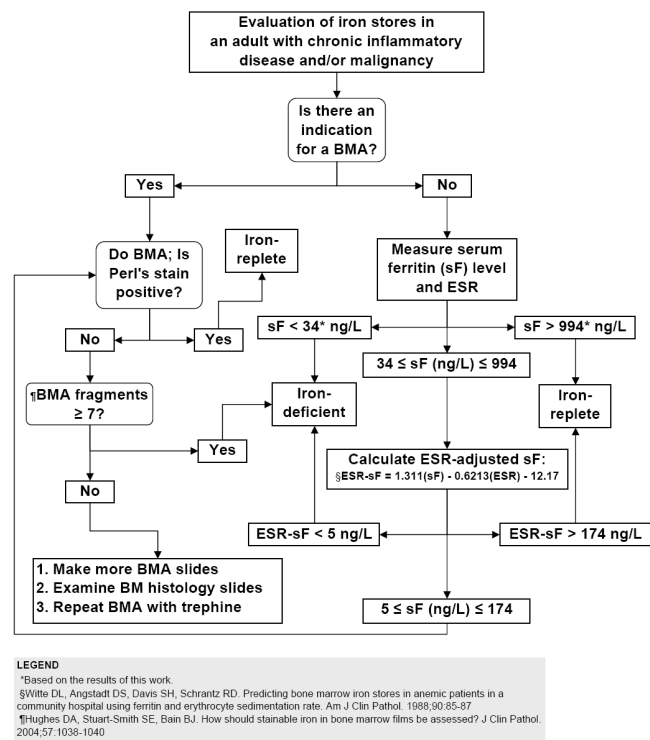

Figure 2. Serum ferritin concentration (sFC) versus ESR-adjusted sFC (z); plotted against negative (PS=0) or positive (PS>=1) Perl's stain.

Figure 3. Algorithm for the evaluation of body iron stores in an adult with chronic inflammatory disease and/or malignancy; in resource-poor settings.

explored the usefulness of CRP, and other non-invasive parameters.(Kurer et al. 1995)

The ESR is particularly attractive in the resource-poor setting as it can be readily done. The effect of ESR-correction on the sFC of this cohort of patients was two-fold. First, the statistical significance of the difference between iron-deficient and iron-replete patients improved significantly from 0.039 to 0.007. Second, the indeterminate serum ferritin range narrowed significantly from 960ng/l (34 - 994 ng/l) to 226ng/l (175 - 401 ng/l). These changes, though yet imperfect, are clear improvements in the bedside utility of these simple tests.

Witte et al(Witte et al. 1988; Witte et al. 1986) using several statistical methods had shown that ESR and serum ferritin were effective in differentiating iron-deficient from iron-replete anaemic patients. Several other studies establishing the diagnostic role of serum ferritin and ESR have been reported.(Charache et al. 1987; Lipschitz 1990; Skikne et al. 1990) Similarly, Ahluwalia et al had reported the positive predictive role of ESR and serum ferritin in identifying iron deficiency in the

face of chronic inflammation in elderly women.(Ahluwalia et al. 1995)

However, some authors have not been able to replicate the successes of these workers. Coenen et al(Coenen et al. 1991) using serum ferritin, CRP, fibrinogen, and ESR in 73 patients with anaemia of chronic disease, found that normograms designed with these parameters were unsuitable for correcting for the acute-phase component of changes in ferritin concentrations.

We conclude that in Nigerian patients with infections and/or malignancies, serum ferritin levels though well above the reference range of the healthy population, do not necessarily correlate with adequacy or otherwise of marrow iron stores in the absence of correction. However, serum ferritin concentration of < 23 ng/l consistently predicted iron-depleted marrows.

Our work suggests that other measures of acute phase reactions such as the CRP, plasma transferrin receptor and fibrinogen, not affected by changes in iron metabolism, that have been shown to synergistically improve the predictive utility of the serum ferritin need to be further evaluated in the developing world to further clarify their role. The cost and sustainability of these tests need also to be re-examined to prove their relevance in these settings. Ultimately, we believe that an optimal strategy that will be easy to deploy and cost-effective to

implement is urgently needed to improve patient management and reduce the frequency of unnecessary marrow aspirates in the tropical patients with co-morbidities.

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